

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, wherein the flow channel comprises:

a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and

a nucleic acid synthetase immobilized in the regeneration region,

wherein the ~~nucleic acid synthetase has an optimum~~ temperature of the regeneration region is controlled at 30 to 40°C.

Claim 2 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid amplifier further comprises a means for controlling temperature, wherein the means for controlling temperature is capable of heating the denaturation region and of keeping a temperature of the regeneration region lower than a temperature of the denaturation region.

Claim 3 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 4 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 5 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 6: (Cancelled)

Claim 7 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 8 (Previously Presented): The nucleic acid amplifier of claim 1, further comprising a solution-sending device for directionally regulating a flow of the reaction solution, wherein the solution-sending device is controllable to periodically reverse the direction of flow of the reaction solution.

Claim 9 (Currently Amended): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising:

(a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;

(b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the ~~nucleic acid synthetase has an optimum~~ temperature of the regeneration region is controlled at 30 to 40°C.

Claim 10 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 11 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 12 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 13 (Previously Presented): The nucleic acid amplifier of claim 3, wherein the flow channel passes at least once the denaturation region followed by the regeneration region.

Claim 14 (Previously Presented): The nucleic acid amplifier of claim 4, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claims 15-18 (Cancelled)

Claim 19 (Previously Presented): The nucleic acid amplifier of claim 2, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 20 (Previously Presented): The nucleic acid amplifier of claim 3, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 21 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the flow channel passes each of the regeneration region and the denaturation region from 20 to 40 times.

Claim 22 (Previously Presented): The nucleic acid amplifier of claim 21, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least each regeneration region.

Claim 23 (Previously Presented): The nucleic acid amplifier of claim 3, wherein two or more different nucleic acid synthetases are immobilized on the beads.

Claim 24 (Previously Presented): The nucleic acid amplifier of claim 4, wherein two or more different nucleic acid synthetases are immobilized on the inner wall of the regeneration region.

Claim 25 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the nucleic acid synthetase is immobilized on an inner wall of the flow channel along the entire length of said flow channel.

Claim 26 (Previously Presented): The nucleic acid amplifier of claim 1, wherein the flow channel comprises a semi-permeable capillary, wherein a medium of a thermostatic chamber,  
on which the capillary is mounted, is a reaction substrate comprising the phosphate compound and the metal ion to supply the reaction substrate continuously into the capillary.

Claim 27 (Previously Presented): The nucleic acid amplifier of claim 22, wherein a portion of the denaturation region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 12 mm, wherein a portion of the regeneration region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm, and wherein a portion of the bead-filling part in one flow channel unit has the width of 1000  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm.

Claim 28 (Currently Amended): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, wherein the flow channel comprises:

a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and

a nucleic acid synthetase immobilized in the regeneration region,

wherein the temperature of the regeneration region ~~has an optimum temperature of~~ is controlled at 30 to 40°C.

Claim 29 (Currently Amended): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising:

(a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;

(b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the temperature of the region in which the regeneration region is controlled at  
~~is performed has an optimum temperature of 30 to 40 °C.~~

Claim 30 (Currently Amended): A nucleic acid amplifier comprising at least one flow channel therein, wherein a reaction solution comprising at least a nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion is caused to flow through the flow channel and to thereby perform nucleic acid amplification in the flow channel, e wherein the flow channel comprises:

a denaturation region wherein a denaturation reaction is carried out, the denaturation reaction comprising melting the intramolecularly formed, the intermolecularly formed, or the intermolecularly and intramolecularly formed double strand of the nucleic acid template;

a regeneration region wherein a double strand is formed with the nucleic acid template, after the double strand thereof is melted, and the nucleic acid primer; and

a nucleic acid synthetase immobilized in the regeneration region,

wherein the ratio in volume between the regeneration region and the denaturation region is about 7:1, and

wherein the temperature of the regeneration region is controlled at 30 to 40 °C.

Claim 31 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the nucleic acid amplifier further comprises a means for controlling temperature, wherein the means for controlling temperature is capable of heating the denaturation region and of

keeping a temperature of the regeneration region lower than a temperature of the denaturation region.

Claim 32 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 33 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 34 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 35 (Cancelled)

Claim 36 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the flow channel comprises a circulation flow channel, the circulation flow channel comprising the regeneration region and the denaturation region.

Claim 37 (Previously Presented): The nucleic acid amplifier of claim 30, further comprising a solution-sending device for directionally regulating a flow of the reaction solution, wherein the solution-sending device is controllable to periodically reverse the direction of flow of the reaction solution.



Claim 38 (Currently Amedned): A method of amplifying a nucleic acid template in a reaction solution comprising at least the nucleic acid template, a nucleic acid primer, a phosphate compound, and a metal ion, comprising

(a) denaturing the nucleic acid template by melting the intramolecularly formed double strand, the intermolecularly formed double strand, or the intramolecularly and intermolecularly formed double strand thereof at a predetermined region;

(b) regenerating a double strand by forming the double strand between the melted nucleic acid template obtained in (a) and the nucleic acid primer at a region different from the region of (a); and

(c) contacting the reaction solution during, just after, or during and just after (b) with a nucleic acid synthetase immobilized and retained in an active state at a region including the region on which (b) is performed,

wherein the ratio in volume between the regeneration region and the denaturation region is 7:1, and

wherein the temperature of the regeneration region is controlled at 30 to 40 °C.

Claim 39 (Previously Presented): The nucleic acid amplifier of claim 31, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least the regeneration region.

Claim 40 (Previously Presented): The nucleic acid amplifier of claim 31, wherein the nucleic acid synthetase is immobilized at least on an inner wall surface of the regeneration region.

Claim 41 (Previously Presented): The nucleic acid amplifier of claim 31, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claim 42 (Previously Presented): The nucleic acid amplifier of claim 32, wherein the flow channel-passes at least once the denaturation region followed by the regeneration region.

Claim 43 (Previously Presented): The nucleic acid amplifier of claim 33, wherein the flow channel comprises at least one unit comprising the denaturation region followed by the regeneration region.

Claims 44-47 (Cancelled)

Claim 48 (Previously Presented): The nucleic acid amplifier of claim 31, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 49 (Previously Presented): The nucleic acid amplifier of claim 32, wherein the flow channel comprises a circulation flow channel comprising the regeneration region and the denaturation region.

Claim 50 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the flow channel passes each of the regeneration region and the denaturation region from 20 to 40 times.

Claim 51 (Previously Presented): The nucleic acid amplifier of claim 50, wherein the nucleic acid synthetase is immobilized on beads, and wherein the beads fill at least each regeneration region.

Claim 52 (Previously Presented): The nucleic acid amplifier of claim 32, wherein two or more different nucleic acid synthetases are immobilized on the beads.

Claim 53 (Previously Presented): The nucleic acid amplifier of claim 33, wherein two or more different nucleic acid synthetases are immobilized on the inner wall of the regeneration region.

Claim 54 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the nucleic acid synthetase is immobilized on an inner wall of the flow channel along the entire length of said flow channel.

Claim 55 (Previously Presented): The nucleic acid amplifier of claim 30, wherein the flow channel comprises a semi-permeable capillary, wherein a medium of a thermostatic chamber, on which the capillary is mounted, is a reaction substrate comprising the phosphate compound and the metal ion to supply the reaction substrate continuously into the capillary.

Claim 56 (Previously Presented): The nucleic acid amplifier of claim 51, wherein a portion of the denaturation region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 12 mm, wherein a portion of the regeneration region in one flow channel unit has the width of 200  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm, and wherein a portion of the

Application No. 10/564,060  
Reply to Office Action of October 24, 2007

bead-filling part in one flow channel unit has the width of 1000  $\mu\text{m}$ , depth 200  $\mu\text{m}$ , and length 25 mm.